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Effects of VX on Acoustic Startle Response and Acquisition of Operant Behavior in Rats

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14. ABSTRACT

This study evaluated the dose-response effects of subacute exposure to sublethal doses of VX on acoustic startle response (ASR) and operant behavior of rats. ASR baseline was established and rats were exposed to 2 consecutive daily doses of fractions (0.2, 0.4, and 0.6) of the established LD_{50} of VX (16 μg/kg). ASR testing continued for 4 days post-exposure. Subsequently animals were dietary restricted and trained to lever press for food under an autoshaping procedure; then a series of escalating fixed ratio (FR) schedules was introduced. Four sessions were conducted under a differential reinforcement of low response rate 10" (DRL 10 s) schedule. Performance under an ascending and descending series of geometric progressive ratio schedules was evaluated. VX decreased the magnitude of ASR on injection days to 100-dB pulses for all exposed groups and to 120-dB pulses only for the 0.4 and 0.6 LD_{50} groups. VX increased the latency to peak startle magnitude in animals receiving 0.4 and 0.6 LD_{50} on injection days. There were no significant dose-related effects on prepulse inhibition and no significant dose-related differences on the acquisition of lever pressing via autoshaping, lever pressing under escalating FR schedules, responding under DRL 10 s schedules of reinforcement, nor responding under geometric progressive ratio schedules. These data indicate few persistent effects of subacute VX exposure on the acquisition of operant behavior.

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VX; chemical warfare nerve agent; acoustic startle response; operant behavior; fixed ratio; autoshaping; differential reinforcement of low response rate; progressive ratio; prepulse inhibition

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ABSTRACT

The present study evaluated the dose-response effects of subacute exposure to sublethal doses of the organophosphorus (OP) chemical warfare nerve agent (CWNA) VX on the acoustic startle response (ASR) and operant behavior of rats. ASR baseline was established and rats were exposed to 2 consecutive daily doses of fractions (0.2, 0.4, and 0.6) of the established LD_{50} of VX (16 µg/kg). ASR testing continued for 4 days post-exposure. Subsequently animals were dietary restricted and trained to lever press for food under an autoshaping procedure. Following the establishment of lever pressing, a series of escalating fixed ratio (FR) schedules was introduced. Thereafter four sessions were conducted under a differential reinforcement of low response rate 10" (DRL 10 s) schedule. Finally, performance under an ascending and descending series of geometric progressive ratio schedules was evaluated. VX decreased the magnitude of the ASR to 100-dB pulses on injection days for all exposed groups and to 120-dB pulses on injection days only for the 0.4 and 0.6 LD_{50} groups. Additionally, VX increased the latency to peak startle magnitude in the animals receiving 0.4 and 0.6 LD_{50} on injection days. There were no significant dose-related effects on prepulse inhibition. There were also no significant dose-related differences on the acquisition of lever pressing via autoshaping, lever pressing under escalating FR schedules, responding under DRL 10 s schedules of reinforcement, nor responding under geometric progressive ratio schedules. Taken together, these data indicate that there are few persistent effects of subacute VX exposure on the acquisition of operant behavior.

INTRODUCTION

VX (0-ethyl S-(2-(diisopropylamino)ethyl)methylphosphonothioate) is a highly toxic organophosphorus (OP) compound used exclusively as a chemical warfare nerve agent. Like other OPs, VX is a potent inhibitor of cholinesterase and produces its toxic effects by excessive accumulation of acetylcholine due to the sequestration of the enzyme responsible for its degradation [31]. Central nervous system (CNS) effects of nerve agents in humans include giddiness, anxiety, restlessness, headache, tremor, confusion, failure to concentrate, convulsions, respiratory depression, and respiratory arrest [25]. There is considerable interest in developing an understanding of the potential neurotoxic effects of sublethal exposure to OP compounds. This interest is especially relevant given their potential use in acts of terrorism, but more importantly to aid the development of more efficacious prophylactic and antidotal compounds.

There are few reports of the behavioral and/or psychological effects of repeated sublethal/subclinical exposure to VX (for a history of human research with nerve agents, see ref. [44]). There is, however, a single report of the behavioral effects of VX in humans [5]. Bowers and colleagues evaluated the physiological and psychological effects of dermal application of VX in 93 human volunteers. The volunteers were administered a profile of mood states (POMS) before and after exposure to VX. Subjects that experienced the most profound psychological, behavioral, and toxic manifestations of poisoning had whole blood cholinesterase levels ranging from 10 - 40% of baseline levels. These subjects were described as being more anxious, experienced greater degrees of psychomotor depression and intellectual impairment, and were more likely to report the occurrence of unusual dreams. Post-exposure, the volunteers whose cholinesterase was most profoundly inhibited evaluated themselves as being less friendly, energetic, and clear thinking than they were pre-exposure. Furthermore, these individuals had more anxiety and greater depression post-exposure as compared to pre-exposure. Another early report that examined the pharmacokinetics of VX and sarin as well as the reactivating efficacy of the oxime 2-pyridinium aldoxime methochloride (2-PAM) in human volunteers also gathered data on the performance of mathematical reasoning [45]. The authors reported decrements on the Number Facility test (a test of simple arithmetic, see ref. [14]) only during the first hour following iv administration of 1.5 µg/kg VX.

The evaluation of the behavioral effects of VX in non-humans has been a relatively ignored area. There are several reports on the efficacy of pretreatments and antidotes to VX poisoning; however, there has been only one publication evaluating the behavioral effects of exposure to VX using animal models [18]. These authors reported on the behavioral and biochemical effects of an acute sub-lethal VX exposure in rats via inhalation. The authors evaluated the rats' behavior post-exposure for approximately 3 months on a previously learned task (Variable Interval [VI] 56" schedule of reinforcement) and on the acquisition of a spatial task (radial arm maze [RAM]). The authors reported inconsistent dose related effects on the performance of VI responding and acquisition of RAM performance in the first two weeks following a single inhalation exposure to a range of concentrations (0.02 – 0.62 LC_{50}) of VX.

Despite the paucity of experimental data on the behavioral effects of VX in particular, there are numerous studies on the behavioral and cognitive effects of other OP chemical

warfare agents (e.g., sarin, soman). Haggerty et al. [20] exposed rats acutely to doses of soman that ranged from 81 – 150 µg/kg (im, 0.5 – 1.0 LD_{50}) and at 2 hr post-exposure evaluated spontaneous motor activity, fore- and hind-limb grip strength, thermal sensitivity (paw-lick latency), rectal temperature, acoustic startle response, and one-way avoidance responding. Their results indicated that thermal sensitivity and paw-lick latency were affected at doses as low as 100 µg/kg, whereas spontaneous motor activity and avoidance responding were affected at doses at or above 123 µg/kg, and acoustic startle response was affected only at the highest dose tested (150 µg/kg). It should be noted that, for the acoustic startle response, these authors were only measuring pre-pulse inhibition; however, there were no tone-only trials available to compute percent pre-pulse inhibition measures.

Philippens and colleagues [33-35,37] have evaluated the efficacy of prophylactic and antidotal compounds in mitigating the behavioral effects of soman exposure in guinea pigs and marmosets. These studies indicate that for both guinea pigs and marmosets, startle reactions increase following exposure to soman. However, it appears there are species differences between rodents with regard to the effects of cholinesterase inhibition on startle response. In rats and mice [10,11,21,24], startle reactions decrease in response to acetylcholinesterase (AChE) inhibition, whereas in guinea pigs startle reactions tend to increase in response to AChE inhibition [8,13,33-36,46].

There have also been numerous reports of the effects of the OP chemical warfare agents soman (GD) and sarin (GB) on learning or acquisition in non-humans. Several different authors have reported that exposure to GD or GB in doses ranging from 0.5 – 2.0 LD_{50} resulted in deficiencies in the acquisition of step-down [41] or lever-pressing [17] avoidance, differential reinforcement of low-rate (DRL) responding [28], delayed alternation [29], two-choice discrimination reversals [30], and performance in a variety of mazes [6,9,13,15,16,38-40]. Those studies utilized rats, mice, guinea pigs or marmosets as experimental subjects.

In light of the abundance of reports on the disruption of the startle reflex and the acquisition of operant behavior by chemical warfare agent exposure, the present studies were conducted to evaluate the effects of repeated sub-lethal exposure to VX on performance and cognition in a variety of behavioral procedures. We were interested in determining the effects of repeated sublethal exposure to the chemical warfare agent VX on the following: (a) acoustic startle response and pre-pulse inhibition during exposure and within the first week following exposure [10,11,46], (b) the acquisition of lever-pressing for food reinforcement using an autoshaping procedure post-exposure [47], and (c) behavioral transitions [19,32]. Within the last category, behavioral transitions, we were interested in evaluating (i) the development of fixed ratio schedule performance [12], (ii) the development of DRL schedule performance [28], and (iii) finally the performance of progressive ratio behavior [22]. The dose range of VX (0.2 - 0.6 LD_{50}) was chosen based on the experience of the experimenters and previous reports, with the expectation that the lower doses would fail to produce signs of cholinergic toxicity, whereas the highest dose would produce moderate signs of intoxication [1].

METHODS

Subjects

Thirty-two adult male Sprague-Daley rats (initial weights: mean 296 g, range 256 - 324 g) were obtained from Charles River Laboratories (Kingston, NY). Upon arrival they were quarantined for 5 days and observed for evidence of disease. Animals were housed individually in polycarbonate cages in a temperature (21 ± 2 ℃) and humidity (50 ± 10%) controlled colony room maintained on a reversed 12-h light-dark cycle with lights off at 0900 h. All experimental manipulations were conducted during the dark phase of the light-dark cycle. Food and water were available ad libitum in home cages. Animals were implanted subcutaneously (sc) with sterile transponders (IPTT-200; BioMedic Data Systems Inc., Seaford, DE) for animal identification. Animals were allowed to acclimate to the colony room (>1 week) before experimental procedures began. Following the completion of acoustic startle testing, animals were placed under caloric regulation which consisted of feeding the animals an amount of food equal to 90 percent of daily estimated energy requirements (112 kcal / Body Weight $^{0.75}$) [48], at least 1 hour following operant test sessions; water continued to be available ad libitum in home cages.

Apparatus

Acoustic Startle Response

The acoustic startle responses (ASR) were measured in eight commercially purchased startle response chambers (Hamilton Kinder, Poway, CA, USA). Each sound attenuated chamber was equipped with a pizeoelectic accelerometer attached to a Plexiglas base for the transduction of animal movements (calibrated daily for accuracy and adjusted to 1.0 ± 0.02 N). During testing sessions, the animal's movements were restricted by their placement in clear Plexiglas restraints (8.9 x 17.8 cm with an adjustable ceiling set to 8.0 cm). Auditory stimuli were presented through a loudspeaker mounted 24 cm above the animal. A modified Realistic sound level meter (Hamilton Kinder, Poway, CA, USA), with the microphone placed in the location of the subject's head, was used to calibrate the sound pressure level (SPL).

Operant Testing Apparatus

Operant testing was conducted in eight commercially available operant conditioning chambers (Med-Associates, Model ENV-007). Each chamber was enclosed in a ventilated, light- and sound-attenuating cubicle and equipped with two retractable response levers (requiring approximately 0.22 N to operate), an opening centered between the levers through which 45-mg food pellets (Bio-Serv, Frenchtown, NJ, Product #F0165) could be delivered, and a cue light above each lever. Following the completion of autoshaping (see below), the left retractable lever was removed and a fixed lever was installed in its place. The food trough contained an infrared emitter-detector pair for monitoring entries and a light that could separately illuminate the trough. Illumination of the chamber was accomplished via a house light mounted on the wall opposite the response levers. White noise and tones were generated from a speaker located beneath the house light. Reinforcement contingencies and data collection were accomplished with 0.01" resolution using a computer running MED-PC IV® software (Med-Associates, Georgia, VT).

Behavioral Procedures

Acoustic Startle Response

Subjects were placed individually in a chamber and allowed to acclimate to the apparatus one session per day for three days prior to VX exposures with the final acclimation session serving as the pre-exposure baseline. Each session began with a 3-min adaptation period with an ambient noise level of 60-dB SPL (full spectrum, 2 – 40 kHz). Following the adaptation period, 10 each of six unique trials were presented in randomized blocks; each trial was separated by a 15 ± 5 s inter-trial interval (ITI). The six trial types employed were: 120-dB noise bursts alone or with prepulse, 100-dB noise bursts alone or with prepulse, 70-dB prepulse-only trials and no stimulus (60-dB ambient noise). Prepulse trials consisted of a 20 ms burst of 70-dB white noise presented 100 ms before a 40 ms burst of the startle eliciting stimulus (100- or 120-dB white noise). Pulse-only trials consisted a 40 ms (1-2 ms rise/fall time) burst of white noise (60-, 70-, 100-, and 120-dB). The 60- and 70-dB stimuli were stimulus control conditions presented to ensure that there was not significant activity within the recording chamber during testing and to ensure that the 70-dB stimulus alone did not elicit a startle reflex. Each animal's movement was measured for a period of 200 ms following the onset of the test stimulus. The peak startle amplitude (V_{max}) was recorded as the highest observed force occurring during the 200 ms measurement window. The latency to peak startle amplitude (T_{max}) was the time that V_{max} occurred following the test stimulus onset. The amount of prepulse inhibition (PPI) produced was calculated following behavioral testing and equaled the difference in startle magnitude between the pulse-alone and the prepulse plus pulse trials, divided by the startle magnitude for the pulse-alone trials, multiplied by 100. A total of seven acoustic startle response testing sessions were conducted. Table 1 shows the order of behavioral testing across the entire experiment including the number of sessions for each condition and the days post-exposure the testing occurred.

Autoshaping

Following the completion of acoustic startle testing, all animals were placed under controlled feeding as described above for seven calendar days before commencing autoshaping. Subjects were trained to lever press using an autoshaping procedure (as described by [7]). Briefly, each session consisted of 50 trials separated by 35 s (average; range 15 to 55 s) ITIs. Each trial began with the insertion of the left retractable lever and simultaneous illumination of the left cue light. If the animal depressed the lever with sufficient force to register a response within 15 s of its insertion a food pellet was delivered. If the animal failed to register a lever press, the left lever was retracted, the left cue light extinguished, a single food pellet was delivered, and the next ITI initiated. Sessions lasted approximately 45 min in the absence of lever presses. Subjects were run under these conditions for a total of 5 sessions. If, after the 5^{th} session, an animal had made less than 10 lever presses in a single session additional sessions were conducted during which the animal was trained to lever press by the method of "successive approximations" (i.e., shaping) before initiating the fixed ratio transitions (see below). There were 12 animals that required manual shaping to complete lever press training (three control animals, one 0.2 LD_{50} animal, five 0.4 LD_{50} animals, and three 0.6 LD_{50} animals).

Fixed Ratio (FR) Transitions

After all animals had acquired lever pressing, a series of increasing fixed ratio (FR) schedules was introduced. Each session lasted for 60 min or 100 reinforcer deliveries, whichever occurred first, and began with the onset of the house light, 72-dB white noise, and illumination of the left cue light. Each FR value was in effect for 3 consecutive sessions. The FR values were, in order of occurrence, 1, 5, 25, 75, and 5.

Differential Reinforcement of Low Rate Responding (DRL)

Following the last session of the FR transition phase, a differential reinforcement of low rate responding (DRL) 10 s schedule was implemented for 4 sessions. Under this schedule, reinforcer delivery was contingent upon the current response occurring at least 10 s after the previous response (or the beginning of the session). Thus, with the exception of the first response, only inter-response times ≥ 10 s produced reinforcer delivery. Each session began with the onset of the house light and 72-dB white noise and lasted 60 min or 100 reinforcer deliveries, whichever occurred first. A total of four DRL 10 s sessions were conducted.

Progressive Ratio (PR) Transitions

After the completion of the DRL phase, a series of geometrically escalating PR schedule sessions were conducted. Escalation rates of 5, 10, and 20% were used. The initial response requirement was 10. Thus, at an escalation rate of 10%, the response requirement for the first 7 reinforcers was 10, 11, 13, 14, 15, 17, 18, etc. Sessions began with the onset of the house light, left cue light, and 72-dB white noise and lasted until one of the following conditions were met: 1) 60 min had elapsed, 2) 10 min had elapsed without a lever press, or 3) 100 reinforcer deliveries had occurred. A total of 6 PR sessions were conducted, 2 at each escalation rate, first in an ascending series followed by a descending series. Break point was defined as the highest ratio requirement completed during an experimental session.

VX Exposure

VX (0-ethyl S-(2(diisopropylamino)ethyl)methylphosphonothioate) was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD) and diluted in sterile saline to achieve the desired concentrations (0.2, 0.4 and 0.6 LD_{50}; LD_{50} = 16 µg/kg). The dilute nerve agent was aliquoted into serum vials, sealed with Teflon septa and stored at -80°C until the day of injection. Injections were administered sc on the right flank at a volume of 0.5 ml/kg body weight. For two consecutive days, VX or saline was administered. Behavioral sessions began 30 min after each injection. Signs of cholinergic toxicity (hyperactivity, fasciculations, excessive salivation and lacrimation) were observed in all of the animals receiving 0.6 LD_{50} and one animal receiving 0.6 LD_{50} died approximately 40 min after the last injection.

Statistical Analysis

Statistical analyses were conducted using SPSS® 12.0 (SPSS Science, Chicago, IL). For each dependent variable a repeated measures analysis of variance (ANOVA) was conducted. If, for a dependent variable, there were violations of the assumption of homogeneity of variance, a logarithmic transformation was conducted (100-dB V_{max}, 120-dB V_{max}, overall response rate [FR], and break point [PR]). For all analyses,

Hyunh-Feldt's procedure was used to adjust for violations of assumptions of sphericity of repeated measures and adjusted *P* values are reported. Main effects of within subject factors were evaluated using Bonferroni's procedure. Main effects of dose were evaluated with Dunnett's procedure. Significant interactions were followed by tests of simple main effects. For all analyses, $\alpha = 0.05$.

RESULTS

Acoustic Startle Response

Peak Startle Amplitude (Vmax)

Activity during the presentation of the 60- and 70-dB stimuli was roughly equal for all groups at approximately 0.1 N (data not shown). Due to violating the assumption of homogeneity of variance, the peak startle amplitude (V_{max}) data from both the 100- and 120-dB trials were log transformed prior to conducting the two-way (dose X session) repeated measures ANOVA. The upper panel of Figure 1 shows V_{max} as a function of post-exposure session for the 100-dB stimulus. As seen in the figure, V_{max} for the control animals was higher than that for the other groups. This was confirmed by the ANOVA as there was a significant main effect of dose [$F(3,27) = 5.23$, $p < .01$] and session [$F(6,162) = 21.42$, $p < .01$] as well as a significant dose X session interaction [$F(18,162) = 5.75$, $p < .01$]. The Dunnett's post-hoc test revealed that all VX groups had significantly lower V_{max} than the control group. The step-down ANOVAs revealed that all VX groups had lower V_{max} during post-exposure session 1 than did the controls; similarly, during post-exposure session 2, the two highest VX dose groups had lower V_{max} than did the controls and during post-exposure session 4, the two lowest VX dose groups had lower V_{max} than did the controls.

The lower panel of Figure 1 shows V_{max} as a function of post-exposure session for the 120-dB stimulus. As seen in the figure, V_{max} values for the control group were higher than those for the two highest VX exposure groups; this was confirmed by a main effect of dose [$F(3,27) = 6.59$, $p < .01$] and by the post-hoc Dunnett's test. Furthermore, the significant dose X session interaction [$F(18,162) = 12.85$, $p < .01$] revealed that during post-exposure sessions 1 and 2, V_{max} values of the 0.4 and 0.6 LD_{50} VX groups were lower than those of the control group.

Latency to Peak Startle Amplitude (T_{max})

The upper panel of Figure 2 shows T_{max} as a function of post-exposure session for the 100-dB stimulus. The ANOVA revealed significant main effects of dose [$F(3,27) = 8.64$, $p < .01$] and session [$F(6,162) = 4.95$, $p < .01$] as well as a significant dose X session interaction [$F(18,162) = 1.99$, $p < .02$]. Dunnett's post-hoc test revealed that the 0.2 and 0.6 LD_{50} groups T_{max} values were above the control group's T_{max} values. The interaction revealed that this effect occurred during post-exposure session 1. However, during post-exposure session 2, the 0.4 and 0.6 LD_{50} groups' T_{max} values were significantly higher than those of the control group. Similarly, during post-exposure session 3, the 0.2 LD50 group had higher T_{max} values than did the control and 0.4 LD_{50} groups.

The lower panel of Figure 2, shows T_{max} as a function of post-exposure session for the 120-dB stimulus. The ANOVA revealed no significant main effect of dose [$F(3,27) = 1.93$, $p > .05$]. However, there was a significant main effect of session [$F(6,162) = 8.86$, $p < .01$] and a significant dose X session interaction [$F(18,162) = 2.2$, $p < .01$]. The interaction revealed that T_{max} for both the 0.4 and 0.6 LD_{50} groups was significantly greater than for the control group during post-exposure session 2.

Prepulse Inhibition (PPI)

Figure 3 (upper panel) shows PPI as a function of post-exposure session for the 100-dB stimulus. As seen in the figure, PPI values for all the groups overlap with the exception of post-exposure session 2. This was confirmed by the ANOVA and an insignificant main effect of dose [$F(3,27) = 0.76$, $p > .05$] and an insignificant dose X session interaction [$F(18,162) = 0.65$, $p > .05$]. There was, however, a significant main effect of session [$F(6,162) = 4.33$, $p < .01$]. PPI during post-exposure session 2 was lower than during sessions 3, 4, and 6.

The bottom panel of Figure 3 shows PPI for the 120-dB stimulus as a function of post-exposure session. As depicted in the figure, there were minimal differences in PPI between the different dose groups [$F(3,27) = 1.81$, $p > .05$]. The one exception is post-exposure session 1; during this sesion PPI values of the 0.6 LD_{50} group were significantly higher than those of the control group [$F(18,162) = 2.28$, $p < .01$].

Operant Behavior

Autoshaping of Lever Pressing

There were no significant main effects of dose [$F(3,27)=1.38$, $P = 0.27$] nor a significant dose X session interaction [$F(12,108)=0.49$, $P = 0.88$] on the number of lever presses emitted during the 5 sessions of autoshaping. There was, however, a significant main effect of session [$F(4,108)=27.3$, $P < 0.001$] indicating that lever presses increased across sessions (data not shown).

Fixed Ratio Transitions

Figure 4 shows overall response rate as a function of fixed ratio requirement. As seen in the figure, the response rates of the 0.6 LD50 group were lower than those of the other groups. However, there was neither a significant main effects of dose [$F(3,27) = 0.56$, $P = 0.64$] nor a significant dose X FR interaction [$F(12,108) = 0.36$, $P = 0.97$] on overall response rate under the different FR schedules. There was a significant effect of FR value on response rates [$F(4,108) = 21.46$, $P < 0.001$]. Response rates under the FR 1 were lower than those of all other FR values except FR 75. Similarly, response rates under the FR 5 replication were higher than those under all other FR schedules.

DRL 10 s Schedule

To evaluate overall performance under the DRL schedule, response efficiency (the ratio of responses to reinforcers: efficiency = 1 when every response is reinforced, lower efficiency indicates better schedule performance) was used as the dependent measure (data not shown). There were no significant main effects of dose [$F(3,27) = 0.38$, $P = 0.77$] nor a significant dose X session interaction [$F(9,81) = 1.04$, $P = 0.41$]. There was

a significant main effect of session [$F(3,81) = 72.23$, $P < 0.001$], indicating that responding became more efficient across sessions.

Progressive Ratio Schedule Transitions

Figure 5 shows break point as a function of escalation rate. As seen in the figure, break points for the control group were generally higher than those of the VX groups. The main effect of dose [$F(3,27) = 2.79$, $P = 0.06$] , however, just failed to reach traditional levels of statistical significance. There was also no significant dose X escalation rate interaction [$F(6,54) = 0.70$, $P = 0.64$] on break point. There was a significant main effect of escalation rate [$F(2,54) = 45.29$, $P < 0.001$] indicating that break points increased as the escalation rate increased.

DISCUSSION

The present investigation was designed to examine the immediate and potentially persistent effects of repeated sub-lethal exposure to VX on the behavior of rats. To this end, we were able to identify behavioral effects of this compound on the acoustic startle response when testing occurred 30 min post-exposure; however, we were unable to detect a systematic dose-response relationship on the acquisition of operant behavior and subsequent behavioral transitions.

Effects of VX on Acoustic Startle

VX disrupted the acoustic startle response of animals administered doses ≥ 0.4 LD_{50} only at 30 min post-exposure. There was no disruption of the startle response when tested at times greater than 24 h post-exposure. These results are in general agreement with earlier studies conducted using the chemical warfare nerve agent soman in rats [20] and the carbamate physostigmine in mice [11,24] in that administration of the cholinesterase inhibitor decreased the magnitude of the startle response. The main effect of dose on V_{max} for the 100-dB stimuli should be interpreted cautiously since the V_{max} of the control animals increased by approximately 50% during the first post-exposure session. It appears that the effect of dose may be due more to the movement of the control group from their baseline than to a general decrement of the VX exposed group. Although it could be argued that this is, in itself, a manifestation of toxicity in that the exposed groups didn't show a similar drift. The drift from baseline for the control animals is also evident in the upper panel of Fig. 2, which depicts T_{max} for the 100-dB trials. There is a similar shift in PPI (Fig 3.) at 100-dB, although it is not as pronounced as that for the raw measures.

Effects of VX on operant behavior

The inability to discern effects of VX on the acquisition of operant behavior in the present investigation is in contrast to previous reports on the effects of the nerve agent soman on the acquisition of a variety of tasks [6,9,13,15-17,28-30,38-40]. One obvious explanation for the lack of effects on the acquisition of operant behavior from the present study is that we used lower doses (≤ 0.6 LD_{50}). Many of the other studies were designed to evaluate either the effects of an acute exposure to lethal doses (≥ 1.0 LD_{50}) or the efficacy of prophylactic or antidotal compounds in mitigating the effects of supra-lethal doses.

Another potential explanation for the lack of effects on operant behavior in transition is that none of the surviving animals experienced seizures, although all animals receiving the 0.6 LD_{50} dose had at least 1 sign of cholinergic toxicity following testing (~ 1 h post-exposure) on the second day of exposures. It is well-known that the severity of behavioral deficits following nerve agent exposure is positively related to seizure duration [28], which is also positively related to the severity of neuropathology [2,27]. It has been previously reported that there appears to be a threshold dose (≥ 0.6 LD_{50} soman) for producing neuropathology and behavioral deficits [28]. VX was reported to have the lowest incidence of seizure development among 6 nerve agents in a guinea pig model [42]. The same report indicated that seizures induced by VX were terminated by lower doses of anticonvulsant drugs than were those induced by the other nerve agents under investigation.

From a pharmacological perspective, VX has greater affinity and selectivity for acetylcholinesterase (AChE) than the G-agents [31,45]. However, the time course of inhibition of red blood cell (RBC) AChE is generally slower following intoxication with VX than with the G-agents [4]. VX has the lowest LD_{50} among the conventional nerve agents [42], the slowest rate of "aging" (dealkylation) ($t_{1/2}$ ~ 48 h) of the conventional nerve agents, and is most readily reactivated by oxime therapy [3,23,26]. Poisoning by VX is the most responsive (of the conventional nerve agents) to standard treatment regimens (atropine, 2-PAM, diazepam).

VX has a pharmacokinetic profile that is substantially different from the G-agents resulting in slower onset of seizures in pretreated and untreated animals [42,43]. It would appear that while VX is more acutely toxic than the other conventional nerve agents it is less likely to induce seizures and hence produce neuropathology and behavioral abnormalities in surviving animals (with, albeit, fewer surviving animals). In an effort to evaluate the anticonvulsant efficacy of atropine sulfate, Shih and McDonough [43] pretreated rats with HI-6 and challenged with nerve agent 30 min later. When challenged by VX at 1.6 LD_{50}, none of the animals experienced seizures; however, when pretreated with 2-PAM and challenged by the same dose, seizures occurred in 33% of the animals. In an effort to determine the ED_{50} for seizure termination, the researchers evaluated atropine efficacy when 2-PAM was administered immediately after VX challenge and found the occurrence of seizures to be similar to that in animals pretreated with 2-PAM. A third experiment was conducted to generate seizures following challenge with VX where 2-PAM was administered after the onset of seizure activity. Under these conditions 62% of the animals seized and the other 38% of the animals died before seizures developed. The authors also reported that the time to onset of seizures in animals challenged with VX was ~ 20 min. For comparison, the reported time to onset of seizures with other conventional nerve agents was ~ 4 min from the same study.

In summary, the present results indicate that there are few persistent effects of repeated sublethal exposure to VX at least under the conditions examined in the present experiments. This general finding is in agreement with the only other published report on the behavioral effects of VX in non-humans [18].

Table 1. Sequence of phases, conditions, number of sessions, and the post-exposure day of testing.

Phase	Condition	Schedule	Number of Sessions	Post-Exposure Day*
ASR	Acclimation		2	
	Baseline		1	
	Exposure		2	1-2
	Post-Exposure		4	3-8
Food Restriction				8-14
Operant Acquisition	Autoshaping/ Lever Press training		7	15-24
	Fixed Ratio	1	3	25-29
		5	3	30-32
		25	3	35-37
		75	3	38-42
		5	3	43-45
	DRL -10 s		4	46-51
	Progressive Ratio	5%	1	52
		10%	1	53
		20%	1	56
		20%	1	57
		10%	1	58
		5%	1	59

* Post-exposure days were counted from the first day of exposure.

FIGURE CAPTIONS

Figure 1. Upper panel shows startle response magnitude (V_{max}) across sessions for the different dose groups in response to a 100-dB white noise burst. Post-exposure session 0 represents the baseline session. A single exposure to 0.6 LD_{50} VX decreased startle magnitude at 30 min post-exposure. Similarly, 2 consecutive days of exposure to either 0.4 or 0.6 LD_{50} VX decreased the startle response to 100-dB stimuli. Lower panel depicts V_{max} in response to 120-dB stimuli across post-exposure sessions. A single exposure to either 0.4 or 0.6 LD_{50} VX resulted in decreased startle response magnitude. A second exposure to these doses further reduced the startle response. Ordinate units are Newtons. * denotes significantly different from control group.

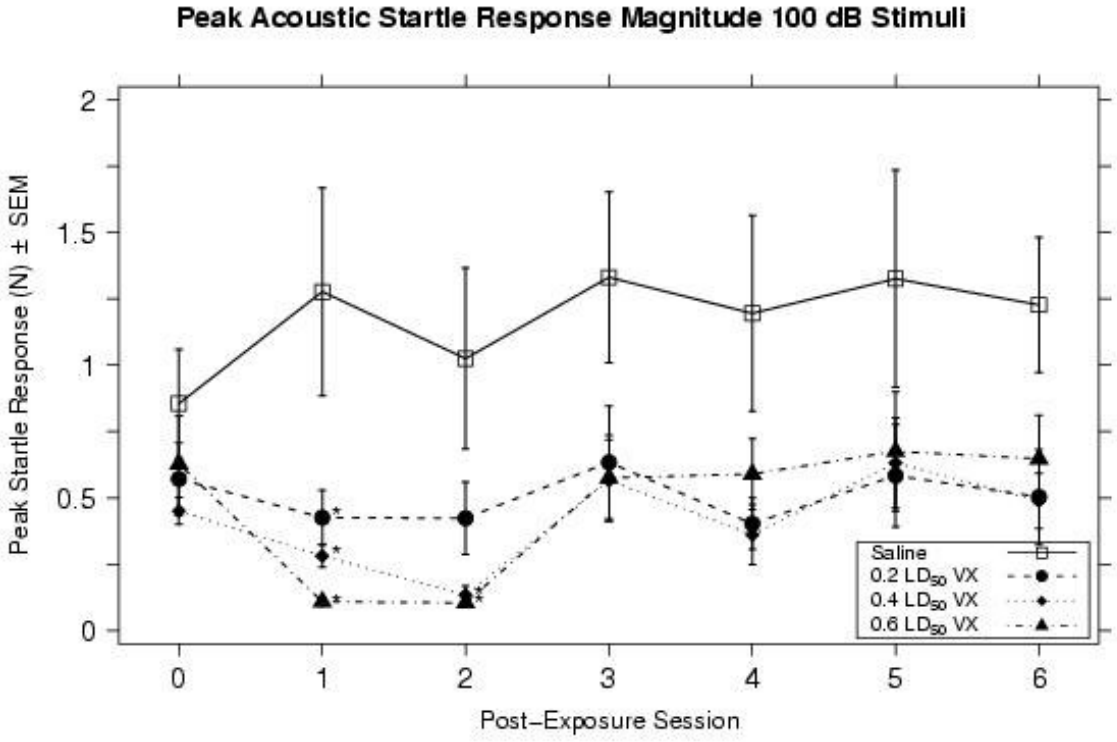
Figure 2. Upper panel shows latency to peak startle response (T_{max}) across post-exposure sessions in response to 100-dB stimuli. During post-exposure session 1, animals exposed to either 0.2 or 0.6 LD_{50} VX had longer latencies to peak startle than did the control animals. A second exposure to 0.4 or 0.6 LD_{50} VX resulted in T_{max} values above those of the control group. Lower panel shows T_{max} in response to 120-dB stimuli. T_{max} values for the 0.4 and 0.6 LD_{50} groups were significantly greater than those of the control group during post-exposure session 2. * denotes significantly different from control group.

Figure 3. Upper panel shows percent prepulse inhibition (PPI) in response to 100-dB stimuli with a 70-dB prepulse stimulus. PPI for the 0.6 LD_{50} group was near 5% during post-exposure session 2; however, this failed to reach statistical significance. Lower panel shows PPI in response to 120-dB startle stimulus with 70-dB prepulse stimulus. PPI for the 0.6 LD_{50} group was significantly greater than that of the control group during post-exposure session 1. * denotes significantly different from control group.

Figure 4. Overall response rate as a function of fixed ratio (FR) schedule requirement. Each point represents the mean of 3 sessions conducted at each FR value. Response rate was biphasic in response to increasing FR requirements. A return to lower FR requirements resulted in increases in response rate. There were no significant differences between dose groups or a significant interaction between dose and FR requirements.

Figure 5. Break point as a function of escalation rate under geometrically incrementing progressive ratio (PR) schedules of reinforcement. Each point represents the mean of 2 replications conducted at each escalation rate, first in an ascending series followed by a descending series.

Figure 1


Peak Acoustic Startle Response Magnitude 100 dB Stimuli
Peak Startle Response (N) ± SEM
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

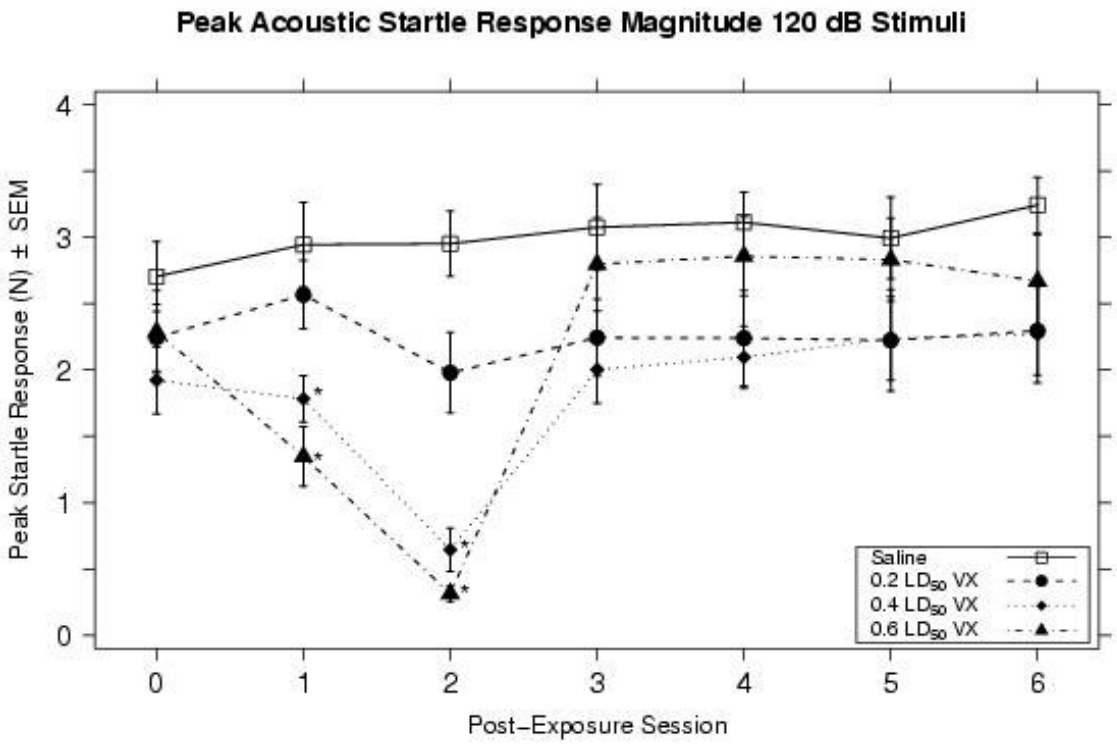

Peak Acoustic Startle Response Magnitude 120 dB Stimuli
Peak Startle Response (N) ± SEM
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

Figure 2

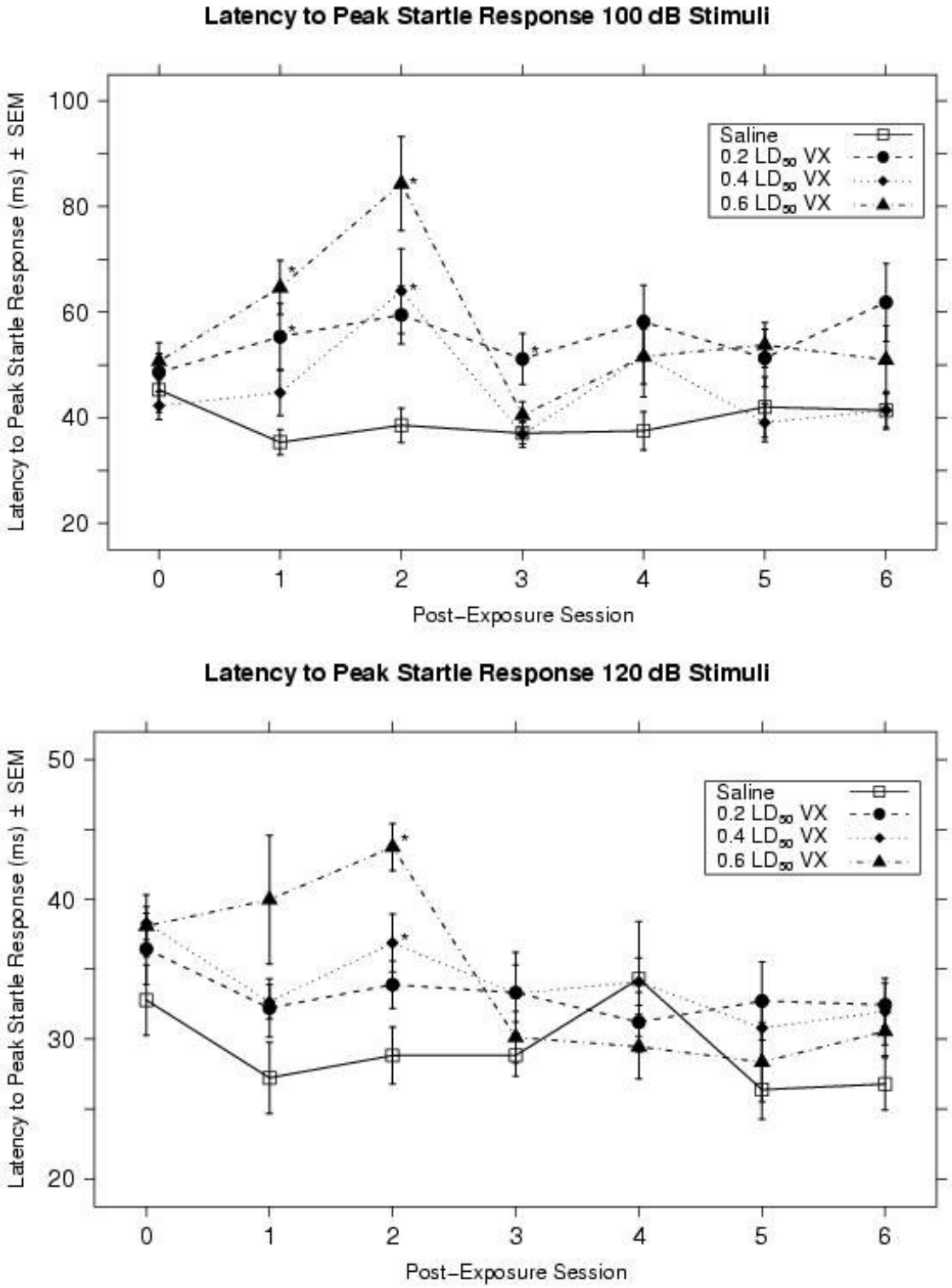

Latency to Peak Startle Response 100 dB Stimuli
Latency to Peak Startle Response (ms) ± SEM
100
80
60
40
20
0
1
2
3
4
5
6
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX
Latency to Peak Startle Response 120 dB Stimuli
Latency to Peak Startle Response (ms) ± SEM
50
40
30
20
0
1
2
3
4
5
6
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

Figure 3

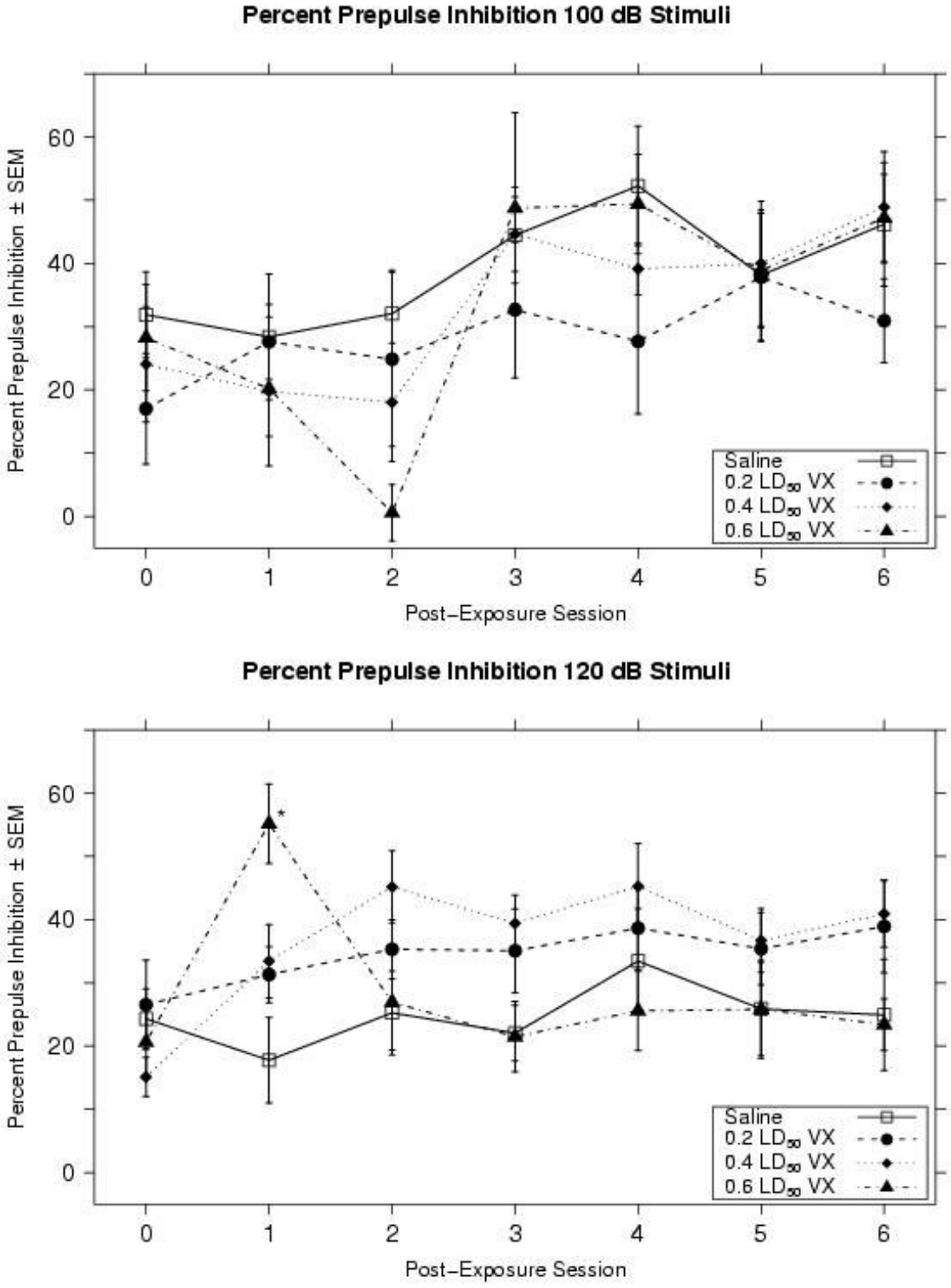

Percent Prepulse Inhibition 100 dB Stimuli
Percent Prepulse Inhibition ± SEM
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX
Percent Prepulse Inhibition 120 dB Stimuli
Percent Prepulse Inhibition ± SEM
Post–Exposure Session
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

Figure 4

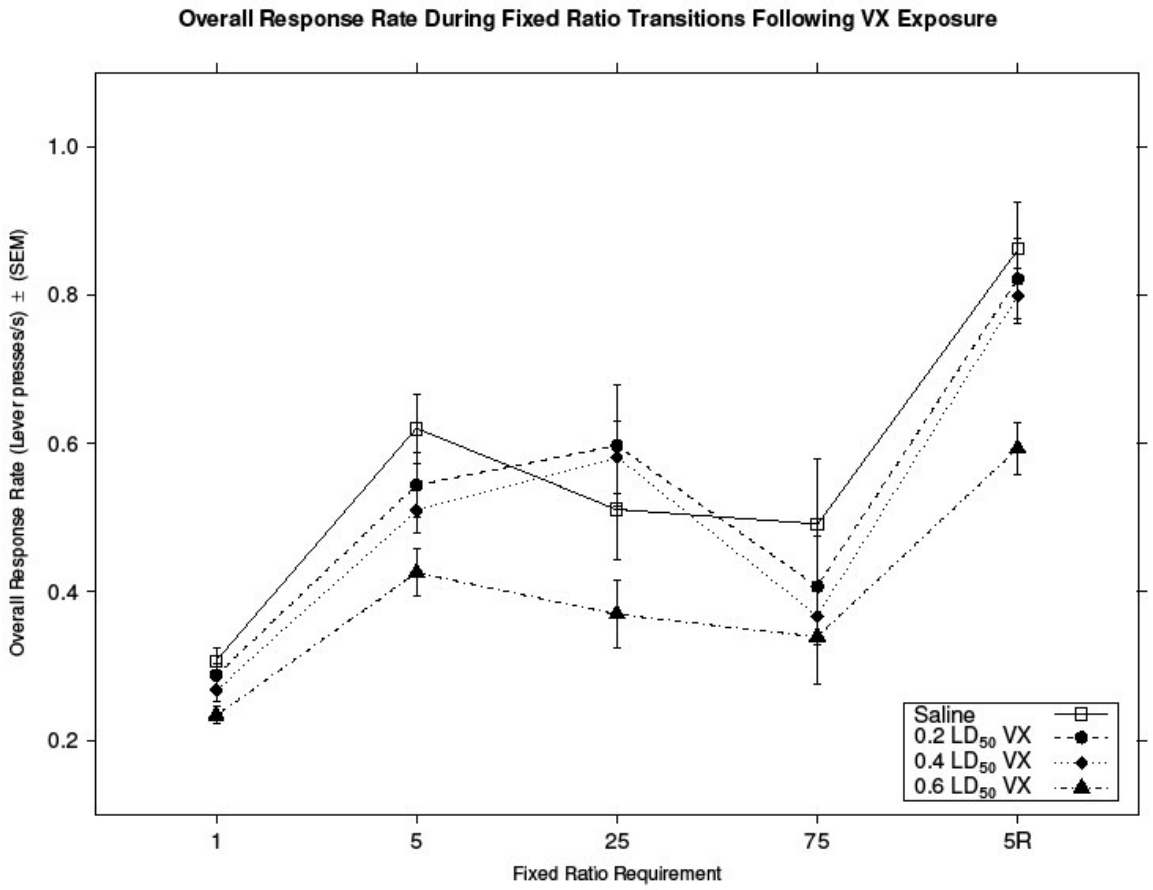
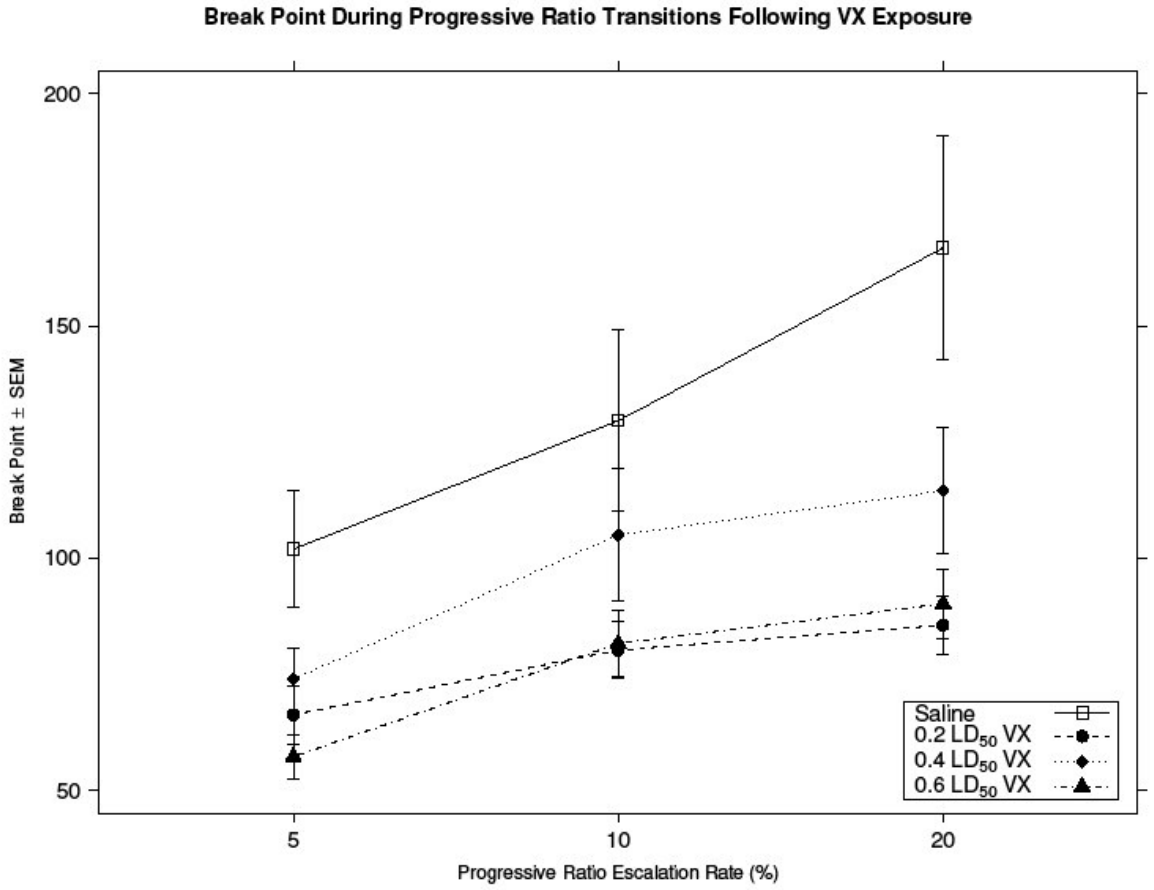

Overall Response Rate During Fixed Ratio Transitions Following VX Exposure
Overall Response Rate (Lever presses/s) ± (SEM)
1.0
0.8
0.6
0.4
0.2
1
5
25
75
5R
Fixed Ratio Requirement
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

Figure 5


Break Point During Progressive Ratio Transitions Following VX Exposure
Break Point ± SEM
200
150
100
50
5
10
20
Progressive Ratio Escalation Rate (%)
Saline
0.2 LD50 VX
0.4 LD50 VX
0.6 LD50 VX

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